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Concentration and intervals of hydrogen dioxide applications to control *Puccinia hemerocallidis* on daylily

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ABSTRACT

Hydrogen dioxide (H_2O_2) is a disinfestant used to kill fungal spores, such as urediniospores of *Puccinia hemerocallidis*, on plant and production surfaces. Excised sections of daylily leaves with sporulating rust pustules were sprayed with concentrations between 19 and 270 g active ingredient (a.i.) $H_2O_2\,l^{-1}$. Treated spores were rubbed onto the surface of PDA, and germination of >100 spores per replication per treatment was determined 24 h later. An exponential decay model was fitted to the data and a lethal dose (LD) of 57 and 114 g a.i. $H_2O_2\,l^{-1}$ solution was predicted to cause 90 and 99% urediniospore mortality, respectively. In an irrigation pad study, one label concentration (2.7 g a.i. $H_2O_2\,l^{-1}$) and approximate LD_{90} and LD_{99} concentrations (54 and 108 g a.i. $H_2O_2\,l^{-1}$) were sprayed 1 or 2 times per week on healthy daylily plants exposed to naturally dispersing inoculum. Disease incidence and severity decreased with increasing concentration and number of applications of H_2O_2 per week. H_2O_2, at 108 g a.i. l^{-1} applied once per week, provided control equal to a fungicide treatment (azoxystrobin and chlorothalonil plus thiophanate-methyl rotation), but was also significantly phytotoxic. In a greenhouse study, two concentrations (2.0 and 2.7 g a.i. $H_2O_2\,l^{-1}$) registered for application on plants and one concentration (3.4 g a.i. $H_2O_2\,l^{-1}$) registered for application on production surfaces were sprayed 2, 3, and 5 times per week on healthy daylily plants exposed to naturally dispersed inoculum. In greenhouse experiment I where the maximum mean incidence was 59%, 2.7 and 3.4 g a.i. $H_2O_2\,l^{-1}$ applied 2–5 times per week provided control equal to the fungicide treatment. In greenhouse experiment II where the maximum mean incidence was 89%, 5 applications of H_2O_2 at 2.0–3.4 g a.i. $H_2O_2\,l^{-1}$ provided control that was better than the water treatment but not as good as the fungicide treatment. Higher label concentrations (2.7 g a.i. $H_2O_2\,l^{-1}$) and multiple applications per week ($\geq$5) can provide disease control equal to a fungicide when disease pressure is low, but may not be effective when disease pressure is high.

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1. Introduction

Hydrogen dioxide (H_2O_2) is one of the few disinfestants registered for direct application on plants (ZeroTol, Environmental Protection Agency (EPA) registration number 70299-1). The ZeroTol label states a list of ornamental plants that are not sensitive to those concentrations. Additionally, the product has been shown to be safely applied at label concentrations at 3 d intervals for a total of five applications to several herbaceous and woody ornamental plant genera (Copes et al., 2003). The use of disinfestants to control foliar disease provides a chemical option with low human toxicity and a zero re-entry interval whereby plants can be treated immediately prior to handling by workers.

Traditional bactericides and fungicides typically have re-entry intervals of 4–48 h, but provide the benefit of a residual protection that is indirectly indicated on pesticide labels by the application intervals, which range from 3 to 21 d. Extensive research has been done to achieve the current knowledge of efficacious usage of bactericides and fungicides with the least number of applications.

In contrast, very limited information pertains to the use pattern of disinfestants to mitigate disease development on plants. Use patterns (intervals between repeat applications) of disinfestants are likely to be dissimilar to those of traditional fungicides. Most studies with disinfestants have evaluated concentration or/and the duration of submersion for a single application on production surfaces (Beuchen and Marth, 1977; Bundgaard-Nielsen, 1996; Copes, 2004). Disinfestants provide an immediate topical activity that targets the bacterial cells or fungal spores of pathogens present on plant surfaces at the time of application but they are not reported to provide a measurable residual protection period. Disinfestants in general react rapidly with inorganic and organic molecules when mixed in a solution or applied to a surface. As a result, the active compound of the disinfestant can be consumptively reduced to a sub-effective dose. This action is

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termed a demand load (Block, 1991). This can be countered by increasing the dose to a level that compensates for the demand load so it does not fall below the effective dose within the time period necessary to kill microorganisms.

A single application of H_2O_2 has been shown to cause mortality of fungal spores on seed surfaces (Allen et al., 2004; Barnett and Varela, 2004). However, H_2O_2 has been shown to provide poor to no control of a number of foliar diseases in chemical comparative trials with weekly applications (Hausbeck et al., 2002, 2005; Schilder et al., 2002; Sconyers and Hausbeck, 2005; Wegulo and Vilchez, 2006). Because H_2O_2 has been shown to provide poor to no control in some circumstances yet is commonly used by commercial ornamental plant producers for disease control, the overall objective of this research was to critically evaluate concentration and interval components of H_2O_2 usage. The research was performed with a heteroecious rust pathogen, *Puccinia hemerocallidis*, that produces the uredial and telial stages on daylily (Hernandez et al., 2002). Urediniospores are produced in a polycyclic pattern and are the primary spore type that causes secondary infection on susceptible daylily cultivars in the southern and southeastern United States (Li et al., 2007; Mueller et al., 2003). To determine if a basic use pattern could be established to control daylily rust, a step wise approach was taken by breaking the overall objective into three objectives, as follows.

1. The first objective was to calculate a lethal-dose curve response and calculate the critical dose at which 90 and 99% urediniospore mortality (a lethal dose) resulted. It has been shown that production surfaces were effectively disinfested of viable *Botrytis cinerea* spores at doses associated with the lethal dose that resulted in $\geq$90% spore mortality (Copes, 2004). This step would establish an experimental-use-concentration that could be used to test product effectiveness and establish the proximity of label concentrations to a lethal dose.
2. The second objective was to evaluate the registered and lethal-dose concentrations when applied at weekly intervals and twice a week in a field trial. This step would evaluate whether differences in efficacy occurred in response to concentration applied at weekly intervals.
3. The third objective was to evaluate label concentrations applied multiple times per week in a greenhouse trial. This step would evaluate whether differences in efficacy occurred in response to application intervals with label concentrations.

2. Materials and methods

2.1. Assessment of urediniospore germination

Hemerocallis cv. 'Pardon Me' was naturally infected with *P. hemerocallidis*. Leaf surfaces and rust pustules were gently scraped with a sterile rubber scraper to remove the majority of urediniospores from the leaf and promote production of new spores. Seventy-two hours after scraping leaf surfaces, 1.5 cm lengths of leaf (replication unit) were cut from infected plants with sterile scissors. Urediniospores were gently dispersed from pustules across the abaxial leaf surface with a sterile rubber scraper. Leaf sections were placed abaxial side up in aluminum trays and sprayed with H_2O_2 under an airflow hood using a Kontes Reagent Sprayer flask (250 ml) with pressurized breathable air at 35 kPa. Treatments were different concentrations of H_2O_2 (ZeroTol, 270 g a.i. l^{-1}, Biosafe Systems, Glastonbury, CT) per 100 ml total solution of deionized water (diH_2O) (Table 1). A completely randomized experimental design was used with four replications, distinguished by independently mixed solutions and individual leaf sections. After leaf surfaces had dried (approximately 4 h after being sprayed), abaxial leaf surfaces were gently rubbed across potato dextrose agar (PDA) to dislodge spores onto the agar with

Table 1

Treatments used in three trials to obtain a dose curve response of *Puccinia hemerocallidis* urediniospore mortality to hydrogen dioxide (=H_2O_2) in a bench top trial, and to control daylily rust on *Hemerocallis* cv. 'Pardon Me' plants treated with registered and high H_2O_2 concentrations applied on an irrigation pad and treated with registered H_2O_2 concentrations applied in a greenhouse.

Concentration (g H_2O_2 l^{-1})	Bench top trial					Concentration (g H_2O_2 l^{-1})	No. of applications per week[a]	
	Experiment						Irrigation pad trial	Greenhouse trial
	I	II	III	IV	V			
0.0	A	A	A	A	A	0.0	1	3
18.9	–	A	–	–	–	2.0[b]	–	2, 3, 5
24.3	–	–	A	–	–	2.7[b]	1, 2	2, 3, 5
29.7	–	–	–	A	A	3.4[b]	–	2, 3, 5
37.8	–	A	–	–	–	54.0[c]	1, 2	–
48.6	–	–	A	–	–	108.0[c]	1, 2	–
56.7	–	A	–	–	–	Fungicide[d]	0.5	0.5
59.4	–	–	–	A	A			
67.5	A	–	–	–	–			
72.9	–	–	A	–	–			
75.6	–	A	–	–	–			
89.1	–	–	–	A	A			
94.5	–	A	–	–	–			
97.2	–	–	A	–	–			
118.8	–	–	–	A	A			
135.0	A	–	–	–	–			
192.5	A	–	–	–	–			
270.0	A	–	–	–	–			

[a] Respective numbers of applications per week (and weekly schedule) were 1 (Monday), 2 (Monday, Thursday), 3 (Monday, Wednesday, Friday), and 5 (daily Monday to Friday).

[b] Product registration lists concentrations of 2.0 and 2.7 g H_2O_2 l^{-1} for curative application on plants and 3.4 g H_2O_2 l^{-1} for application on production surfaces.

[c] Lethal doses associated approximately with 90 and 99% urediniospore mortality (54.0 and 108.0 g a.i. H_2O_2 l^{-1}, respectively) as determined in the *in vitro* experiment.

[d] Azoxystrobin (Heritage 500 g a.i. l^{-1} product, Syngenta, Greensboro, NC), at 0.46 ml a.i. l^{-1}, was applied at the start of each experiment and chlorothalonil plus thiophanate-methyl mixture (Spectrum 90 WDG, 720 and 180 g a.i. l^{-1} product, respectively, Syngenta, Greensboro, NC), at 0.43 and 0.11 g a.i. l^{-1}, respectively (experiments I and II of the irrigation pad study and experiment I of the greenhouse study), or propiconazole (Banner Maxx, 143 g a.i. l^{-1} product, Syngenta, Greensboro, NC), at 0.07 ml a.i. l^{-1} (experiment II of the greenhouse study), was applied 14 d later (=0.5 times per week).

minimal disruption of the agar surface. Approximately 24 h later, more than 100 urediniospores were assessed as germinated or not germinated. The experiment was performed five times because concentrations were adjusted in successive experiments to obtain a more precise calculation of the dose resulting in 90 and 99% urediniospore mortality. The complete treatment selection tested in experiment IV was repeated in experiment V.

2.2. Irrigation pad assessment with daylily plants

Disease-free plants of *Hemerocallis* cv. 'Pardon Me' were obtained from a northern Tennessee nursery. Plants were split and repotted with one to two fans per pot in 3.8 l pots. Additionally, plants of *Hemerocallis* cv. 'Pardon Me' that were naturally infected with *P. hemerocallidis* were obtained from a nursery located in southern Mississippi. This experiment was done on an outdoor irrigation pad versus in a greenhouse to provide increased ventilation as a safety precaution against the possibility of higher than normal volatile gas densities associated with the intentional use of high H_2O_2 concentrations (Table 1). One disease-free and one naturally infected plant were placed together per treatment in a randomized complete block design with four blocks (replications). Treatments within a block were placed in an east-to-west orientated row with two trays of large zinnia plants (50 cm tall) providing a partial physical barrier between treatments. Blocks were orientated parallel to each other and separated by a distance of 5 m. Southern winds are prevalent thus more likely to cause a block versus treatment effect.

Treatments included a factorial treatment selection of H_2O_2 concentration and number of H_2O_2 applications per week, a non-control treatment (diH_2O), and a standard control treatment (fungicide) (Table 1). Treatments were applied for four weeks using a CO_2 sprayer at 176 kPa with a nylon TeeJet 8002VS nozzle. Plants were watered with an overhead center-pivot sprinkler, at approximately 10.00–11.00 h daily. Disease intensity and phytotoxic severity were assessed at the end of week five only from plants that were initially rust-free. Disease incidence was assessed as the number of leaves with one or more rust pustules per the total number of leaves per plant. Disease and phytotoxicity severities were assessed as percent leaf area per plant exhibiting symptoms or signs of daylily rust using Key No. 1.2 as a guide (James, 1971). The experiment was repeated, and chemical treatments started on June 21 and August 17, 2004, respectively.

2.3. Greenhouse assessment with daylily plants

Hemerocallis cv. 'Pardon Me' plants naturally infected with *P. hemerocallidis* in 3.8 l pots were pruned to approximately 1.3 cm above the soil line and sprayed with chlorothalonil and thiophanate-methyl mixture to obtain disease-free plants. Plants were grown in a greenhouse and monitored two to three times a week for rust pustules. Individual leaves were removed upon detection of a uredium initial. If needed, an additional application of a chlorothalonil and thiophanate-methyl mixture was applied, with a minimum interval of 3 weeks before starting an experiment. A set of infected plants were maintained in a separate greenhouse.

Treatments included a factorial treatment arrangement of H_2O_2 concentration and number of H_2O_2 applications per week, a diH_2O control treatment, and a standard control treatment (fungicide) (Table 1). Plants were arranged in six column and ten row positions on a 1.8×6.1 m greenhouse bench and treatments were completely randomized with five replications (plants) per treatment. Six to nine plants naturally infected with *P. hemerocallidis* were interspersed through the treatments on the upwind side of the bench. Air movement was generated from two 51×51 cm house fans set at low speed and positioned at bench height. The experiment was repeated, with the difference that treatments were applied for 5 and 4 weeks in experiments I and II, respectively. For the first 2 weeks of experiment I, plants were watered by hand over the top of the plants. In week three and thereafter in experiment I and throughout experiment II, plants were watered by hand as needed and additionally watered with overhead sprinklers for 10 s at 20 min intervals between 06.00 and 07.00 h. Disease intensity was assessed 6 d after the last treatment application. Disease incidence was assessed as the number of leaves with one or more rust pustules per total number of leaves per plant in experiments I and II. Disease severity was assessed as the percent leaf area exhibiting symptoms or signs of daylily rust on a per plant basis in experiment I. Because plants were larger and disease incidence higher in experiment II, disease severity was assessed on 10 randomly selected leaves. Leaves with representative severity levels were used as a guide.

2.4. Statistical analysis

All statistical analyses were conducted using the SAS software package (version 9.1; SAS Institute Inc., Cary, NC). Urediniospore germination (as a proportional number of germinated urediniospores per total number of urediniospores assessed) and H_2O_2 concentration (g a.i. l^{-1}) was fitted to an exponential decay model using the NLIN procedure. The exponential decay model was selected as more appropriate than linear regression models based on the probability of significance of F tests. The nonlinear model equation was of the form:

$$y = \alpha + \beta \exp(-\delta x),$$

where y is the urediniospore germination for more than 100 spores (dependent variable), x is the H_2O_2 concentration (g a.i. l^{-1}) (independent variable) and α, β, and δ are parameters (α is the asymptote of the minimum possible value of y, β is the unit change in y when x changes from 0 to ∞, and δ is the concentration of change) (Hinds and Milliken, 1987). An F test to detect differences between individual experiments was constructed from regression analyses of the five experiments fitted singly and pooled. A lethal dose of H_2O_2 was estimated by predicting y to be equal to a 0.90 and 0.99 reduction below the maximum value of y (equivalent to a 90 and 99% urediniospore mortality, respectively).

For all *in vivo* experiments, analysis of variance was performed using the MIXED procedure (SAS version 9.1.3, Cary, NC). A randomized complete block experimental design was used in the irrigation pad experiments. A completely randomized design was used in the greenhouse experiments. Fixed effects included two control treatments (water non-control and standard fungicide control) and a factorial treatment design of H_2O_2 concentrations, number of H_2O_2 applications per week, and the interaction between concentrations and the number of applications per week. Random effects were experiment and replication. An orthogonally balanced Mixed model was constructed using coding to partition the control treatments (=Ck) and the factorial comparison of hydrogen dioxide concentrations and applications per week. The model structure allows direct comparison of the factorial design of H_2O_2 treatments (concentration, number of applications, and the interaction) and a separate comparison between the non-control (water) and standard control (fungicide) treatments. Pair-wise comparison of concentration and number of applications of H_2O_2 were assessed from t-tests for the statistical significance ($P \leq 0.05$) of the difference between least square means (LSMEANS). Estimate statements were used to assess pair-wise comparisons between individual means of H_2O_2 concentrations or number of applications and individual control treatments. Each dependent variable (rust incidence, rust severity, and phytotoxicity) was modeled separately.

3. Results

3.1. Urediniospore germination

Experiments were not significantly different (at $P < 0.05$) so data from all five experiments were pooled. Parameter estimates were $\alpha = 0.011$, $\beta = 0.278$, and $\delta = 12.389$ for the exponential decay model. Since α, the intercept term, was not significantly different from zero based on 95% confidence limits of −0.0231 and 0.0457, it was set to zero. Concentrations considerably higher than the concentration (2.7 g a.i. H_2O_2 l^{-1}) listed on the product registration were required to achieve 100% mortality (Fig. 1). The lethal dosages (and 95% confidence limits) estimated to cause 50, 90, and 99% urediniospore mortality were 17.2 (11.9, 22.5), 57.2 (39.6, 74.8), and 114.4 (79.1, 149.7) g a.i. H_2O_2 l^{-1}, respectively.

3.2. Irrigation pad trials

Incidence ($P = 0.8082$) and severity ($P = 0.0718$) were not significantly different between experiments, so data from both experiments were combined for each type of assessment. Daylily rust intensity (both incidence and severity) decreased with increased concentrations of H_2O_2, but only incidence decreased with increased applications per week (Table 2). Interaction between H_2O_2 concentration and applications per week was not significant for either incidence ($P = 0.2402$) or severity ($P = 0.2775$) assessments. While most treatments had significantly lower rust incidence than the water treatment (Table 2), only the highest concentration (108.0 g a.i. H_2O_2 l^{-1}) provided disease control comparable to the fungicide treatment (Table 2).

Phytotoxicity symptoms differed significantly between experiments ($P < 0.0001$), therefore experiments were analyzed separately. The 2.7 g a.i. H_2O_2 l^{-1} (registered concentration) produced no plant damage and was equivalent to the water and fungicide treatments (Table 3). A significant interaction occurred due to H_2O_2 concentration and applications per week in experiments I and II ($P < 0.0001$ and $P = 0.0301$, respectively). At 108.0 g a.i. H_2O_2 l^{-1}, phytotoxicity symptoms increased with increased applications per week, but this trend was less consistent with 54.0 g a.i. H_2O_2 l^{-1} (Table 3). Phytotoxicity symptoms resulting from the application of 108.0 g a.i. H_2O_2 l^{-1} were unsightly and not acceptable. Symptoms consisted mainly of interveinal and marginal necrosis with small, 1–3 mm diameter lesions to larger blotchy yellow and necrotic spots.

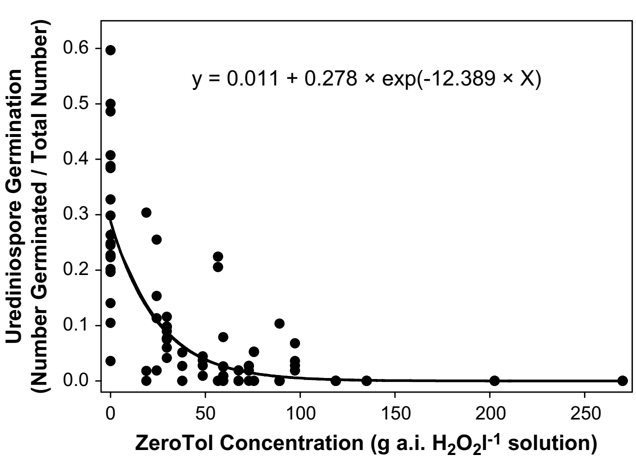

Fig. 1. Proportional number of urediniospores that germinated after exposure to hydrogen dioxide concentrations. Hydrogen dioxide solutions were sprayed on leaf sections, the leaf sections were allowed to dry, spores were gently rubbed across the surface of potato dextrose agar, and spore germination assessed 24 h later. Data were fitted to an exponential decay model (predictive line).

Table 2
Disease incidence and severity assessments of daylily rust on *Hemerocallis* cv. 'Pardon Me' plants treated with hydrogen dioxide (H_2O_2) for 4 weeks on an irrigation pad.

Chemical concentrations (g a.i. H_2O_2 l^{-1})	Incidence (%)	Severity (%)
0 (Deionized water)	86.8 a[a]	8.12 a
2.7	79.1 a	4.72 b
54.0	60.2 b	0.75 c
108.0	36.4 c	0.26 c
Fungicide[b]	38.6 c	0.14 c
Applications per week (chemical)		
1 (Deionized water)	86.8 a	8.13 a
1 (H_2O_2)	64.6 b	1.85 b
2 (H_2O_2)	52.6 c	1.96 b
0.5 (Fungicide)[b]	38.6 d	0.14 c

[a] Analysis of variance was performed using Proc Mixed (SAS version 9.1.3). Treatments with the same letter are not significantly different. Comparison of H_2O_2 concentrations and applications per week were based on differences of least square means ($P = 0.05$). Comparison of individual H_2O_2 treatments and the water or fungicide treatment were based on differences calculated using estimate statements ($P = 0.05$).

[b] Azoxystrobin, at 0.46 ml a.i. l^{-1}, was applied at the start of the experiment and chlorothalonil plus thiophanate-methyl mixture, at 0.43 and 0.11 g a.i. l^{-1}, respectively, was applied 14 d later (=0.5 times per week).

In experiment I, all treatments, even water, exhibited symptoms that were evaluated as a phytotoxic effect. This is evident by the higher ratings in experiment I versus II (Table 3). The plants were not checked for other problems, such as mite infestation, which could have caused yellowing and necrosis. Despite the potential of phytotoxicity symptoms being confounded with an additional problem in experiment I, the only pronounced phytotoxic symptom occurring in both experiments was on plants treated with 108.0 g a.i. H_2O_2 l^{-1} applied twice per week (Table 3).

3.3. Greenhouse trials

Disease intensity of daylily rust was reduced by the application of H_2O_2, but the level of control differed depending on whether incidence or severity was assessed (Table 4). Rust severity was not different between experiments ($P = 0.2611$), while incidence was significantly different between experiments ($P < 0.0001$). Rust incidence, in experiment I, and severity, in experiments I and II, were significantly lower in response to H_2O_2 concentrations

Table 3
Percent daylily leaf tissue exhibiting phytotoxicity symptoms after treatment with hydrogen dioxide (H_2O_2) for four weeks.

Chemical concentrations (g a.i. H_2O_2 l^{-1})	Applications per week	Phytotoxic severity (% plant area)	
		Experiment I	Experiment II
0 (Deionized water)	1	8.8 a[a]	1.1 a
2.7	1	6.6 ab	0.5 a
2.7	2	7.8 ab	0.1 a
54.0	1	4.6 b	2.1 a
54.0	2	7 ab	6.2 b
108.0	1	4.7 b	5.7 b
108.0	2	19 c	11.8 c
Fungicide[b]	0.5[b]	5.6 a	0 a

[a] Analysis of variance was performed using Proc Mixed (SAS version 9.1.3). Treatments with the same letter are not significantly different. Comparison of H_2O_2 concentrations and applications per week were based on differences of least square means ($P = 0.05$). Comparison of individual H_2O_2 treatments and the water or fungicide treatment were based on differences calculated using estimate statements ($P = 0.05$).

[b] Azoxystrobin, at 0.46 ml a.i. l^{-1}, was applied at the start of the experiment and chlorothalonil plus thiophanate-methyl mixture, at 0.43 and 0.11 g a.i. l^{-1}, respectively, was applied 14 d later (=0.5 times per week).

Table 4
Disease incidence and severity assessments of daylily rust on *Hemerocallis* cv. 'Pardon Me' plants treated with hydrogen dioxide (H_2O_2) for 5 and 4 weeks in experiments I and II, respectively, in a greenhouse.

Chemical concentrations (g a.i. H_2O_2 l^{-1})	Incidence (%)		Severity (%)
	Experiment I	Experiment II	
0 (Deionized water)	58.5 a[a]	89.4 a	8.1 a
2.0	20.9 b	83.0 a	4.2 b
2.7	3.3 c	78.3 a	3.3 b
3.4	3.1 c	80.4 a	2.9 b
Fungicide[b]	9.6 bc	5.6 b	2.9 b
Applications per week (chemical)			
3 (Deionized water)	58.5 a	89.4 a	8.1 a
2 (H_2O_2)	12.2 b	85.1 a	4.2 b
3 (H_2O_2)	12.3 b	86.8 a	3.3 bc
5 (H_2O_2)	2.8 b	69.8 b	2.1 c
0.5 (Fungicide)[b]	9.6 b	5.6 c	2.9 bc

[a] Analysis of variance was performed using Proc Mixed (SAS version 9.1.3). Treatments with the same letter are not significantly different. Comparison of H_2O_2 concentrations and applications per week were based on differences of least square means ($P = 0.05$). Comparison of individual H_2O_2 treatments and the water or fungicide treatment were based on differences calculated using estimate statements ($P = 0.05$).

[b] Azoxystrobin, at 0.46 ml a.i. l^{-1}, was applied at the start of the experiment and chlorothalonil plus thiophanate-methyl mixture, at 0.43 and 0.11 g a.i. l^{-1}, respectively (experiment I), or propiconazole, at 0.07 ml a.i. l^{-1} (experiment II), was applied 14 d later (=0.5 times per week).

(2.7 and 3.4 g a.i. H_2O_2 l^{-1}) and number of applications per week (3 and 5), than the water treatment and not different from the fungicide treatment (Table 4). However in experiment II, rust incidence was not different between plants treated with water or each of the H_2O_2 concentrations applied two and three times per week. Only five applications of H_2O_2 per week resulted in a rust incidence lower than the water treatment, but incidence was still considerably higher than the fungicide treatment (Table 4). Rust incidence in the water treatment (untreated control) was higher in experiment II than in experiment I. The interaction between H_2O_2 concentrations and frequency of applications per week were not significant for incidence in experiment I or II ($P = 0.7546$ and 0.4113, respectively) or severity ($P = 0.9526$). Phytotoxic symptoms did not develop in response to the H_2O_2 concentrations and intervals tested in the two experiments performed in the greenhouse.

4. Discussion

Hydrogen dioxide kills urediniospores of *P. hemerocallidis* and can reduce foliar disease development comparable to a fungicide. This appears to be in contrast to results by others (Hausbeck et al., 2002, 2005; Schilder et al., 2002; Sconyers and Hausbeck, 2005; Wegulo and Vilchez, 2006) which indicate that weekly applications of H_2O_2 will not provide satisfactory control of foliar diseases. This research substantiates their findings that weekly applications of label concentrations of H_2O_2 are not likely to provide disease control, but also demonstrates that an increase in concentration or a decrease in interval between applications can result in increased control efficacy.

An increase in concentration is probably the least logical approach to improving efficacy of H_2O_2, because of the potential for increased risk of plant damage. As an example, a weekly application of 108.0 g a.i. H_2O_2 l^{-1} solution controlled rust comparable to a fungicide, but caused severe phytotoxicity to daylily plants. The fact that 54.0 g a.i. H_2O_2 l^{-1} solution (predicted to cause approximately 90% urediniospore mortality) did not cause a reduction in disease comparable to 108.0 g a.i. H_2O_2 l^{-1} solution (predicted to cause approximately 99% urediniospore mortality) indicates that concentrations applied at weekly intervals need to cause a very high spore mortality and that the concentrations determined in the *in vitro* experiment are representative of control activity on the plant. These high rates are not too dissimilar from the rate (81.0 g a.i. H_2O_2 l^{-1} solution) needed to disinfest pine seed surfaces of *Fusarium* spp. (Barnett and Varela, 2004).

A question not addressed in this research is how representative the sensitivity of *P. hemerocallidis* urediniospores are compared with the sensitivity of other fungal pathogens to H_2O_2. Copes (2004) showed that metal, plastic, and wood production surfaces were completely disinfested of *B. cinerea* spores with a concentration of 10–30 g a.i. H_2O_2 l^{-1} solution. Furthermore, five applications of 10 g a.i. H_2O_2 l^{-1} (=10,200 μg g^{-1} a.i. H_2O_2, the highest concentration tested in that trial) at 3 d intervals did not damage the leaves of alyssum and candytuft (Copes et al., 2003). Based on this information, organisms differ in their sensitivity to H_2O_2 and select pathogens may be sensitive enough to be controlled by weekly applications of H_2O_2 concentrations.

If weekly applications of label concentrations of H_2O_2 are not likely to control many foliar diseases and higher concentrations involve increased risk for plant damage, the next logical approach is to evaluate whether shorter intervals could be effective. With multiple applications per week, H_2O_2 did provide control equal to a fungicide treatment, however results were not consistent between experiments. In greenhouse experiment I, H_2O_2 provided disease control comparable to the fungicide, based on both incidence and severity assessments. Control was still evident in experiment II but not at a level that would be acceptable in a commercial production system. Based on incidence assessment in experiment II, five applications of H_2O_2 per week provided rust control that was better than water (non-control treatment) but considerably poorer than the fungicide. Yet based on severity assessment, H_2O_2 limited the buildup in rust severity comparable to the fungicide.

The relationship between incidence and severity may provide some insight into the differences between the two greenhouse experiments. For many foliar diseases including diseases caused by other *Puccinia* spp., incidence initially increases at a much faster rate than severity and only during later stages, after incidence levels are high, do severity levels increase more rapidly (Dillard and Seem, 1990; Imhoff et al., 1982; James and Shih, 1973; McRoberts et al., 2003; Pataky and Headrick, 1988; Seem, 1984). In greenhouse experiment I, disease incidence was observed to be developing slowly, so starting in week 3, overhead irrigation was run for a 10 s duration at 20 min intervals from 06.00 to 07.00 h daily to extend the high humidity and leaf wetness periods that commonly occur during night and morning hours. The maximum incidence in greenhouse experiment I was 59%. The same morning misting schedule was used throughout the entire period of experiment II and maximum incidence was 89%. The supplemental misting appeared to be conducive to rust development, although environmental data and multiple disease assessments needed to quantify these assumptions were not taken. In both greenhouse experiments, H_2O_2 applications provided significant reduction in severity levels. The differences in disease development between greenhouse experiments I and II are evident based solely on incidence assessments, and could be explained by differences in environmental conditions that were conducive to rust development for a shorter time period in experiment I than in experiment II. The results imply that efficacy of H_2O_2 may be lower under high disease pressure conditions, but further investigation is needed before such a conclusion can be verified.

The general concepts of how disinfestants work favours the use of multiple applications per week. Many polycyclic foliar pathogens have multiple dispersal events whereby infective propagules are repeatedly deposited on plant surfaces over time. Infection events are more a function of daily inoculum levels and environmental conditions versus a weekly schedule. In this study, disease pressure was higher than should occur at the start of a commercial crop, since mature diseased plants with active sporulating urediniopustules were set between the apparently disease-free plants. It would be prudent to use good sanitation practices coupled with regular H_2O_2 applications to further minimize disease development.

Frequent H_2O_2 applications, such as daily applications, would be expensive if applied by human applicators. Likely, the most cost effective application method would be as an automated application through the irrigation system. The application of H_2O_2 would need to be timed separate from an irrigation event to water plants, because considerably less water thus less H_2O_2 would be required to wet leaves and stems of the plant versus thoroughly watering the organic rooting media.

Efficacious use of fungicides is the result of many years of research. Disinfestants, such as H_2O_2, should not be sold short based on negative results from simple chemical comparative trials. This research shows H_2O_2 can control disease, but additional research is needed to more completely understand how to use H_2O_2 effectively and when a classical fungicide should be used alternatively.

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